INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK
WAGENINGEN, NEDERLAND
DIRECTEUR: Dr. J. G. TEN HOUTEN

MEDEDELING No 150

NEW ASPECTS OF BREEDING FOR DISEASE RESISTANCE IN BEANS

DOOR

N. HUBBELING

OVERDRUK UIT:
EUPHYTICA, 6: 111–141, 1957

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Euphytica 6 (1957): 111–141

NEW ASPECTS OF BREEDING FOR DISEASE RESISTANCE IN BEANS

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Received 12th Feb. 1957

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1. INTRODUCTION

In the Netherlands as well as in other countries new varieties of beans (*Phaseolus vulgaris* L.) with resistance to some important diseases have been bred in the past 10 years.

Unfortunately it soon appeared that these new varieties were insufficiently resistant against other diseases which were no serious problem with the established varieties.

However, four ways which may assist in remedying the situation soon, can be mentioned:

(a). In Western Europe there is now no uncertainty about the symptoms of the most important bean diseases, and in consequence this represents a basis for selecting for resistance. In order to show how bean diseases can be distinguished eight coloured plates (Figs 1–8) which appeared in other publications of the writer have been included in this paper (13, 14, 15, 16). These illustrations show that in reacting to the same disease different bean varieties display completely different symptoms. Symptoms also vary according to conditions. If the less variable characteristic disease symptoms are constantly kept in mind it is usually not difficult to identify the diseases and the resistance.

(b). Methods which have been developed for testing the resistance of large numbers of plants against five important diseases, can now be employed by private breeders. The division of disease resistance of the Institute for Phytopathological Research (I.P.O.) advises breeders on these testing methods and supplies them with the infection material required. In addition much advice is given on the combined artificial testing for various diseases simultaneously, although often the tests can be carried out successively when, for some reason, it is not possible to carry out a single combined test.

(c). Numerous bean varieties could be evaluated as parent plants by testing seedlings for resistance to, for example, *Phaseolus virus 1* and *2*, *Nicotiana virus 11*, halo blight and anthracnose. The combined testing of hybrid populations has been started and is already being tackled on a large scale by a number of private breeders, the Institute of Horticultural Plant Breeding (I.V.T., Wageningen), the Foundation for Agricultural Plant Breeding (S.V.P., Wageningen), and the Vegetable Research Station at Alkmaar.

(d). As a result more experience is now available about the genetics of resistance in cultivated varieties and wild species, and about cultural and consumption characteristics.

It is very important to combine resistance against diseases mentioned above as soon as possible and without losing other valuable features. In this connection there are some new and unexpected aspects:

2. Disease symptoms as a principal basis for testing for resistance

(a). *Common bean mosaic and black root. Phaseolus virus 1*

Common bean mosaic

Common bean mosaic is a virus disease which may be seed-borne in susceptible varieties, though mostly only to the extent of several percent. In the field the virus is transmitted by winged aphids from diseased to healthy plants. In the central, southern and eastern parts of the Netherlands large numbers of winged aphids of the species *Aphis fabae* often occur, resulting in heavy infestations in most bean fields.

Testing in the field.

Here the testing of bean varieties and hybrid populations can be easily carried out by sowing in the second half of May or at the beginning of June. Some rows of diseased plants, obtained by sowing heavily-infected seed, are laid out alongside the rows to be tested. Resistance can generally be judged prior to or during flowering.

In the coastal areas of the northern part of the Netherlands there are often insufficient winged aphids to cause heavy infestations in the field, even in the middle of the summer. In such cases one is restricted to artificial testing by means of sap inoculation of seedlings.

Artificial testing.

It is necessary to start with diseased plants which are infected with an extremely virulent virus strain. The leaves and stems of such plants are crushed in a mortar with a small quantity of water (e.g. 10 times as much as the weight of the plant material). The sap is filtered through cotton-wool and, after adding carborundum powder of 500 or 600 mesh, carefully rubbed with thumb and forefinger on to the first simple leaves of the plants to be tested. The leaves are then rinsed with water and kept fairly warm – preferably not over 25 °C. At this temperature the first symptoms appear after 10 to 14 days. After 3 to 4 weeks differences in resistance are clearly visible because characteristic symptoms will have by then developed. A rapid method of testing has been developed by Richards and Munger (19b). They used a suction feed glass-atomizer with a Devilbiss cutoff assembly in inoculating large numbers of seedlings.

Susceptibility and resistance.

In the case of very susceptible varieties curling of leaves, chlorosis and subsequent necrosis of the leaf tissues are the typical symptoms of attack. In addition growth is stunted and pod-setting is delayed. Moderately susceptible plants develop rolled leaves with mosaic, and often exhibit slight symptoms of chlorosis; they are also inhibited in growth. Tolerant or slightly susceptible plants grow almost normally but in the first stages of attack they can be recognized by their somewhat narrower leaves with slightly chlorotic veins. Later abnormalities involving apical and lateral shoots do not occur. On the other hand a very high percentage of seed transmission of the virus can sometimes be established in the case of tolerant varieties.

Black root

Bean varieties showing no symptoms of mosaic sometimes develop brownish-black discoloration of vessels within the plant, including the roots. For years the cause of these symptoms, known as black root, could not be discovered, but eventually GROGAN and WALKER (11) found it to be due to hypersensitivity of the plants to *Phaseolus virus 1* at temperatures of 20 °C or higher.

Testing in the field.

The testing for resistance to common bean mosaic and black root combined can be carried out in the field only when temperatures have been sufficiently high (17, 18). Therefore the procedure will be most successful when sowing takes place in the second part of May in a warm protected area in the south. Plants containing common bean mosaic should serve as the source of infection in this case, and should be sown next to the beans to be tested.

In the coastal areas few winged aphids develop on account of the prevailing windy and cool weather, and black root occurs there rarely.

Artificial testing.

In these regions resistance can be tested by grafting mosaic virus containing shoots infected with mosaic virus on to healthy plants in a glasshouse where the temperature does not fall below 20 °C (20, 33). As yet, however, practical breeders do not test their plants by this rather elaborate method, but the development of necrosis in bean seedlings three or four days after sap-inoculation at temperatures above 30°C may be an indication for them how to test lines (28, 29). Even cut leaves in petri dishes show necrotic veins after sap inoculation at these temperatures (19a).

Susceptibility and resistance.

The growing of varieties susceptible to black root has shown that in practice there are great differences in the degree of attack. Varieties which normally develop little foliage and roots, such as the dwarf snap-bean Servus, are frequently heavily attacked, whereas well-rooted, large, leafy plants are only seldom attacked. It appears that bean varieties which remain free from attack by black root in the Netherlands often show hypersensitivity in countries where the temperatures reach higher levels in summer. The disease does not generally make its appearance in the field until the plants have produced mature pods, except in a hot summer like 1947, when many seedlings died.

Environmental conditions.

Temperature is therefore presumably a decisive factor in controlling the incidence of the disease. If the crop is sown late (for example after mid-June) so that pods develop in September, black root does not even occur in very susceptible varieties in the Netherlands. Probably the lower overnight temperatures are sufficient to compensate for possible high afternoon temperatures.

(b). *Yellow bean mosaic and apical necrosis. Phaseolus virus 2*

Unlike *Phaseolus virus 1*, *Phaseolus virus 2* is not seed-borne in susceptible bean varieties. In the field the virus is transmitted by winged aphids from infected gladioli, clover, or other plants. The presence of these winter host plants in the vicinity of beans is therefore a *conditio sine qua non* for an attack, and the degree of attack is greatest where the host plants are closest to the bean crop.

Testing in the field.

Consequently the testing of bean varieties and hybrid populations can be carried out in a simple way by sowing the beans alongside rows of infected gladiolus plants in the second half of May or in early June. Sufficient numbers of winged aphids are necessary, of course, to bring about the infection. In the central, southern and eastern parts of the Netherlands winged aphids sometimes start transmitting the virus as early as May. During the course of June and July large populations are built up and resistance can be tested in the field, because in those months young plants seldom escape infection.

As has already been mentioned, viruses are transmitted by winged aphids only to a very limited extent in the northern coastal provinces, and resistance to *Phaseolus virus* 2 there must be tested artificially.

Artificial testing.

This is carried out by transmitting the virus in diluted sap derived from diseased bean plants on to the simple leaves of the seedlings. The sap, together with fine carborundum powder of 500 or 600 mesh (see page 112) should then be rubbed carefully over these leaves with the aid of a small glass rod or with the fingers. Only wounds of microscopic size should be produced and through these the virus particles penetrate into the intact cells of the epidermis of the plants. Rinsing the plants with water after inoculation prevents the sap from having a toxic effect on the cells. Direct radiation by the sun and high temperatures should be avoided in order to prevent the superficially injured cells from drying up, particularly during the first 24 hours. After 10 to 14 days the first symptoms begin to appear in the rubbed developing leaves. Two to four weeks later the symptoms reach the stage when the degree of resistance or susceptibility of the plants can be assessed.

Susceptibility and resistance.

In the case of very susceptible bean varieties the apical and lateral shoots, and also the young inflorescences, are killed, and growth and podsetting are arrested. Moderately susceptible varieties generally develop yellow leaves which become curly and crisp rather soon. In addition growth is inhibited in the apical and lateral shoots, and

FIG. 9. ABNORMAL SYMPTOMS OF PHASEOLUS VIRUS 2 IN A CROSS OF THE DWARF SNAP BEAN VARIETIES SERVUS AND DUBBELE WITTE Z.DR.:
Old leaf with local vein necrosis; malformed young leaf with vein clearing; pods with superficially necrotic ringspots

Abnormale symptomen van Phaseolus virus 2 in kruising van de stamslabonerassen Servus en Dubbele Witte z.dr.:
Oud blad met plaatselijke nerfafsterving: misvormd jong blad met lichte nerftekening: peulen met oppervlakkige necrotische ringvlekken

the leaves in those regions remain much smaller than normal, being distinguishable by a clearly marked mosaic. Slightly susceptible or tolerant varieties often display a weakly-developed mosaic pattern. This pattern is restricted to the veins or to scattered yellow or reddish brown dots in the leaf tissues (see Fig. 9).

It is dubious whether completely resistant varieties can occur within the species *Phaseolus vulgaris* L. Tolerant bean varieties showing only weak symptoms, at most, appear to suffer no observable damage, so are sufficiently resistant.

(c). *Stipple streak. Nicotiana virus 11* (*tobacco necrosis virus*)

The virus disease designated stipple streak is distinguished from the preceding diseases by:

a. the absence of mosaic symptoms
b. overwintering of the virus in the soil
c. impossibility of transmission by aphids

Characteristic symptoms of stipple streak are reddish brown discolorations in the stems, leaf veins, pods and roots of the plants (19). Discoloration is not restricted to the vessels but extend locally to parts of the cortex and the epidermis.

Testing in the field.

When susceptible varieties are sown in infested soil the lateral roots soon turn brown and die. As a result the plants begin to suffer from a "foot-rot disease" which causes the leaves to turn yellow while the plants are greatly inhibited in growth. Sometimes no red stripes or discoloured leaf veins appear in the aerial parts of the plant, although the virus can be easily found in the roots. The most reliable method for assessing the resistance of the plants to be tested therefore is to examine the root system, provided that it has developed sufficiently in heavily-infested soil. The roots of resistant plants are always vigorous and white coloured; these plants are therefore firmly rooted and continue to grow for a long time to produce a sturdy crop.

Artificial testing.

If there is uncertainty about the degree of contamination of the soil, seedlings and older plants can be tested artificially by using diluted sap from diseased plants. The sap reaches the limit of infection power only at a dilution of 1 to 100,000 (parts by weight). The sap, either by itself or mixed with carborundum powder, is rubbed on to a simple or compound leaf of the plant to be tested. Infection can also be brought about by injecting this sap into stems or pods.

Susceptibility and resistance.

It is some time before one can be sure of the resistance of the plants tested; the appearance or non-appearance of symptoms on inoculated parts of plants is no criterium of resistance for only when no symptoms appear in the other parts, particularly in the roots, stems or pods, can one be certain that the plants are resistant. Although local symptoms often appear after 3 or 4 days, a month or more should elapse before assessing the resistance. Brown discolorations in the pods can sometimes appear in

moderately susceptible bean varieties towards the end of the season. In the case of highly-susceptible varieties the plants die without producing pods, especially if they were infected in the seedling stage. It is not yet certain whether the bean varieties claimed as being resistant are, in fact, tolerant or perhaps hypersensitive to the virus. The fact that they sometimes respond with local lesions seems to be an indication of hypersensitivity.

Environmental conditions.

Stipple streak can attack the plants both at low temperatures and at fairly high temperatures. QUANTZ obtained infection of cut leaves in petri dishes after sap-inoculation at temperatures of 15 °C up to 25 °C. No infection resulted at 30 °C (19a). An attack during a heat wave in summer is sometimes temporarily checked, perhaps also as a result of inactivation of the virus by intensive and long irradiation of the plants by the sun. As a rule the most severe attacks are observed in pole beans, probably since it is difficult for sun rays to penetrate into their foliage. Insufficient crop rotation may also play a part.

The virus remains intact for several months in dry plant material preserved under cool conditions, so it is not difficult to place infection material at the disposal of private breeders and plant breeding institutes at any time. Usually the virus is increased in living green plants in order to have sufficient material for the infection of large numbers of seedlings available for disposal.

(d). *Halo blight. Pseudomonas phaseolicola (Burkh.) Dowson*

Halo blight is the only important bacterial disease of beans in the Netherlands. In the first instance the bacteria are disseminated by sowing contaminated seeds of susceptible bean varieties. The seedlings infected by seed transmission are severely inhibited in growth and turn yellow; sometimes no compound leaves develop at all. It is characteristic that chlorosis is almost entirely restricted to the leaf parenchyma tissues, resulting in a pattern of green veins similar to the symptoms of a virus disease. It is especially conspicuous in young leaves developing in cool weather (temperatures of under 20 °C).

Testing in the field.

The symptoms on plants contaminated in the field by bacteria from rows of natural or artificial infected seedlings are restricted to the parts infected. Under these circumstances chlorotic lesions develop in the centre of which a "greasy" spot marks the presence of bacteria in the intercellular cavities of the leaf. This "greasy" spot dries up in dry warm weather and turns reddish brown in colour; it may fall out of the leaf leaving a small hole. During wet weather the bacteria are spread by rain drops which touch the "greasy" spots. The bacterial exudate then appears as minute creamy-white slime "knobs" on leaves, stems and pods amongst the "greasy" spots. High winds can spread the infected drops over fairly large distances, producing infection centres in other leaves, and by the rubbing of leaves against each other and against the ground. In addition, the bacteria can easily penetrate into leaves through small wounds made by plants rubbing against one another.

Artificial testing.

Four methods are known for artificial testing the resistance of seedlings (or green pods):

1. Rubbing the simple leaves or green pods with an aqueous suspension of bacteria, using fine carborundum powder (500–600 mesh) for producing small wounds in the epidermis, to increase the chance of infection. One practical breeder preferred to use a small roughly polished glass rod for applying the suspension without carborundum powder. In addition he inoculated the first compound leaves because these may be assessed during a longer period. The simple leaves are often discarded by the plants when they are diseased. The degree of resistance can be ascertained from the development of small or large halo blight spots produced in the leaves or pods.

2. Injection of a bacterial suspension into the hypocotyl or epicotyl stem by means of a syringe. In this way the bacteria reach the vessels immediately and, in susceptible plants, symptoms will appear which resemble those on seedlings grown from infected seed. Injection of green pods is possible in the same way.

3. Immersion of freshly germinated seeds in a bacterial suspension for two hours, and then planting them in pots. Before immersing the seeds the seed coat is removed n order to aid the penetration of the liquid into and between the cotyledons. This procedure can be furthered in vacuum (31). Here again symptoms are produced in susceptible plants which resemble those induced by seed transmission of the disease. Green pods can be treated in the same way.

4. Inoculation of beans by atomizing a suspension of bacteria has been most successful at the time the third trifoliate leaf was unfolding. Infection was variable and slight on the primary leaves (24).

The methods mentioned under 1 and 2 can be combined with inoculation for testing for resistance to stipple streak.

Susceptibility and resistance.

There are considerable differences in the rate of multiplication of the bacteria in the plants of different bean varieties and this is closely related to differences in resistance. In very susceptible varieties large "greasy" spots are produced not only in the leaves, stems and pods, but also in the xylem of stems and leaf veins which become filled with bacterial slime. In such cases growth is chlorotic and greatly inhibited not only on account of the actual bacteria in the vessels, but particularly because they secrete toxins (26). Under unfavourable conditions the plants hardly recover, and frequently the leaves and apical stems wither and no pod-setting and seed formation takes place. Moderately susceptible plants also develop "greasy" spots in leaves, stems and pods but they remain more limited in size. Stunting occurs to a lesser degree, but the bacteria can penetrate deeply into the plants infecting the seeds when the pods are attacked. In this case infected seeds may often show a satisfactory germination capacity and thus contribute to the spread of the disease. In slightly susceptible varieties the bacteria multiply only to a small extent in the plants; under unfavourable circumstances small "greasy" spots may occur on leaves and pods, but these will soon dry up and turn brown in sunny weather. Plant development is hardly checked by the bacteria and only slightly chlorotic margins around the "greasy" spots in the

leaves are produced if, at 10–15 °C, normal growth is no longer possible. Slightly susceptible bean varieties are sufficiently resistant to halo blight under normal conditions, and the bacteria are not seed-borne. The highest degree of resistance known to the writer is characterized by the absence of "greasy" spots. The presence of small necrotic spots in areas of infection may often be an indication of this resistance.

Environmental conditions.

With the above mentioned testing methods for resistance, the best results are obtained when the temperature is not allowed to rise much above 20 °C. In the last mentioned methods the temperature should be maintained at between 15 °C and 20 °C so as to induce slight chlorosis in susceptible plants. The first mentioned artificial method is a little less dependent on the temperature because it is not the effect of the toxic substance which is considered in assessing the susceptibility of the plants, but the multiplication of the bacteria in the leaves (32). Even in almost completely resistant bean varieties the toxin can produce chlorosis at temperatures as low as about 10 °C and this would lead to incorrect assessment.

Infection material.

The cultures of bacteria required for testing are supplied by the resistance department of the I.P.O. direct to breeders. However, breeders can also produce the infection material themselves because the bacteria can be preserved in dry plant material for a year at least without impairing its virulence. The easiest way is to collect pods with halo blight in the field, allowing them to dry up and then keeping them in a sufficiently dry condition. For the preparation of a bacterial suspension these dry pods only need to be soaked in tepid water for some hours to activate the bacteria again. Cutting the dry material into small pieces and crushing it in a mortar after soaking will probably result in a considerable number of bacteria being brought into suspension. If dry pods with halo blight spots are not available a sufficient amount of infection material can be prepared with one culture of bacteria. Infection of a number of seedlings of a susceptible bean variety according to the method described under 2 or 3 produces diseased plants. In their stems enormous numbers of bacteria are developed and large quantities of suspension can be produced by crushing the material in a mortar with water or by dispersion in a mixer. By splitting the stems of seedlings lengthwise and washing with water, already a sufficient quantity of bacterial suspension can be obtained. In order to be able to use the suspension for injecting stems it should be filtered through cotton wool to remove coarse particles. When preparing bacterial suspensions great care should be taken to exclude decaying parts of plants since the presence of putrifying bacteria in the diseased parts to be used may kill inoculated seedlings within a short time.

(e). *Anthracnose. Colletotrichum lindemuthianum (Sacc. et Magn.) Scrib.*

When infected seed is sown the resulting seedlings may be seriously attacked and even killed. Symptoms of anthracnose occur in nearly all countries of the world where beans are grown. Sometimes infected seedlings die even before they emerge and this results in a thin stand.

Sunken dark brown spots on the leaf veins and stems at all stages of plant development are characteristic symptoms of anthracnose. Flower stalks and young pods may also be attacked, and eventually brown spots develop on pods and seeds. The fungus spores which are produced in the brown lesions can be spread by rain drops from diseased to healthy plants in the field during cool moist weather. Diseased fields in the vicinity can therefore eventually lead to the infection of a healthy crop.

Field testing.

Owing to the physiological specialization of the fungus it has proved difficult to carry out a reliable test for resistance in the field.

Specialization of the fungus.

On comparing the results of artifical testing of some bean varieties with numerous fungus isolations of Dutch origin it appeared that the cultures could be classified into four categories and in accordance with similar research in the U.S.A. the races are indicated as *alpha*, *beta*, *gamma*, and *delta*. Determination was possible with the aid of a small number of American dry shell beans as appears from table 1 (27, 34, 35).

TABLE 1. SPECIALIZATION OF COLLETOTRICHUM LINDEMUTHIANUM

Bean varieties	Alpha	Beta	Gamma	Delta
Emerson 847	R	R	R	S
Perry Marrow	S	R	S (R)	S
Michelite	S	R	S R	S
Michigan Dark Red Kidney .	R	S	S	S

R = resistant. S = susceptible. (R) = in the U.S.A. a resistant line of Perry Marrow seems to be known.

Only seedlings killed after infection were considered susceptible. A slight attack which sometimes occurred in "resistant" plants was tolerated. Completely resistant plants do not display any symptoms at all.

Artificial testing.

1. In order to test numerous bean varieties for resistance against different isolations of the fungus the seedlings are raised in culture tubes of 4 cm diameter. For that purpose every group of five germinated seeds, after removing the seed coats, is placed at the bottom of a tube on a filter paper folded to form a small table. The seedlings are allowed to grow by adding a small quantity of water to every tube, causing the filter paper to remain wet. The plants are inoculated by atomizing a spore suspension over the seedlings when the hypocotyl starts to lengthen occurring mostly before or just when the lateral roots commence to develop. The tubes are sealed by means of cotton wool plugs for at least three days. If desired cotton wool can be replaced by polyaethylene-plastic. At a temperature of 20 °C, a sufficient number of spores will have germinated and penetrated into the plants within 2 × 24 hours of inoculation. Three days should be allowed to elapse however, at lower temperatures, and maintenance of a high degree of air humidity during the further incubation time appears to be favourable for the development of the fungus in the plants. At 20 °C symptoms

appear about a week after inoculation and some days later very susceptible plants are heavily attacked or dead. This permits selection for resistance to be easily carried out.

Very young parts of mature plants such as inflorescences, pods or lateral shoots may be killed in the field only after cool moist weather; so tubes can also be used successfully for testing young shoots of nearly mature plants.

A private Dutch breeder tested in 1956 the resistance of some 70,000 seedlings in culture tubes according to the method described above. However, in general, this method should be considered of limited use for practical breeders, since it requires some laboratory equipment and a knowledge of laboratory procedure.

The advantages of this method are the following:

a) Seedlings in tubes can easily be placed under the desired temperature conditions in the laboratory.
b) No special preparatory measures are necessary to keep air humidity at the required high level.
c) There is extremely little chance of the seedlings escaping infection, on account of the individual treatment.
d) The seedlings can be treated at about the same stage, since late germinating seeds can be kept a little warmer in seperate tubes for one or more days in order to stimulate growth.

The disadvantages are that:

a) It is not easy to plant out the seedlings at the stage when disease symptoms are visible without damaging the root system. The above-mentioned breeder overcame this problem by transferring the plants from the tubes into pots prior to the appearance of symptoms, obtaining plants with a well-rooted ball of soil which could be planted out in the field, if necessary, under less favourable weather conditions.
b) The work of placing the seeds in the tubes and subsequently carefully taking them out involves much labour, particularly as a considerable number of susceptible plants have to be dealt with.

2. The last-mentioned difficulty led to a search for a method of making tube treatment of the seedlings superfluous. Three private breeders agreed to sow seed in trays or boxes containing, respectively, moist saw dust, moist river sand on top of a layer of wet peat-dust, and in vermiculite. Inoculation was carried out when all the seeds had germinated and to promote uniform germination, the seeds were sometimes cut. A spore suspension was sprayed over the seedlings in the trays at the stage mentioned before, although this still appeared to be successful when the simple leaves between the cotyledons were entirely unfolded. In order to allow inoculation of the seedlings just after germination, the seeds have to be sown superficially. To keep them sufficiently moist they may be covered by wet filter paper or plastic. Later on the trays – seperately or together – were sealed hermetically with polyaethylene-plastic or with glass and after three days air was gradually allowed to enter in order to harden the plants. Sometimes the seedlings were not planted out in the field until differences in resistance to anthracnose were clearly visible. The disadvantage of this procedure, however, was that the plants had difficulty in forming fresh roots. In order to assist root development the plants were often planted in good garden soil in pots

under glass. Since a bean plant can more easily produce new adventitious roots when it is young, best root development can be obtained with seedlings which are potted before they develop disease symptoms. The advantage of well-developed plants almost outweighs the work of potting up numerous plants which later show themselves to be susceptible. In any event a well-developed root system is a prerequisite for good production of pods and seeds.

3. At some plant breeding institutes the testing of seedlings is carried out in water in zinc reservoirs using wire netting and filter paper (11a). This method makes it possible to test large numbers of seedlings in the laboratory. However, it would seem to be a less convenient method for most private breeders, but one used it very successfully.

Regarding the different methods it may be said that every breeder can achieve the best results by using his own particular method. This involves individual experience, for as a rule use must be made of available equipment. It further depends on the intelligence and routine of the available staff as to how many plants can be tested simultaneously. Experienced selectors manage to deal with large numbers of plants with simple means and in a short time. Testing simultaneously some 10,000 plants or more has proved to be quite feasible.

Environmental conditions.

The incubation time required is longer at a lower temperature. At 15 °C the first disease symptoms do not appear until at least ten days have elapsed, and at least two weeks should pass before the results of the testing are assessed. With all methods of testing this low temperature is preferable, because then the seedlings remain in a good condition, allowing them to be transplanted for a rather long time. However, there need not be any doubt about susceptibility and resistance between 14 °C and 22 °C. At higher temperatures susceptible plants are attacked locally in stead of being killed. Nevertheless the optimum temperature for germination of spores is 28 °C (16a).

Susceptibility and resistance.

The specialization of *Colletotrichum lindemuthianum* explains the differences in resistance to the four races of the fungus. In general, bean varieties in the field show a higher degree of resistance if they appear to be resistant to more races of the fungus. The bean varieties which appear to possess no factors or only a single factor for resistance are considered to be extremely susceptible. In the field the apical shoots and young pods often die off and a very high mortality of seedlings may also occur, leading to complete crop failure. If resistance to two races occurs, damage is often restricted to a deterioration in the quality of the pods and seeds harvested. Usually deep-seated lesions do not develop so that the seeds are not damaged severely, but superficial attacks of the seeds often take place.

It is, of course, possible that one race of the fungus causes considerable damage in one place while another does not affect the crop at all in another place. Where there is resistance to three races crop damage in the field occurs only exceptionally, and is normally restricted to superficial spots. Occasionally the seeds are heavily attacked. Plants which are resistant to all four races indeed remain entirely free from

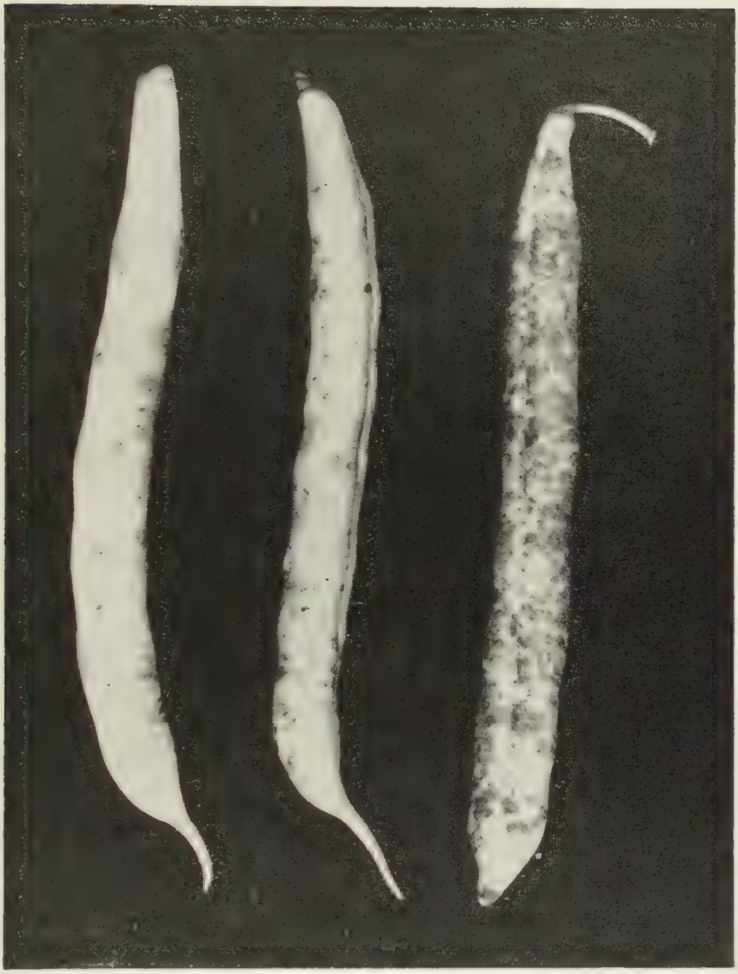

FIG. 10. MATURE PODS OF THE DWARF SNAP BEAN, VARIETY SAXA, SLIGHTLY ATTACKED BY ANTHRACNOSE DUE TO SUPERFICIAL INFECTION LATE IN THE SEASON. Abnormal symptoms

Rijpe peulen van het stamslabonenras Saxa, licht aangetast door vlekkenziekte ten gevolge van oppervlakkige infectie laat in het seizoen. Abnormale symptomen

damage under unfavourable conditions. In breeding beans it is therefore desirable to select for this highest degree of resistance and if this does not occur in a hybrid population, not all the races of the fungus can be used for the test. Every race is maintained and increased separately at the I.P.O. and can be supplied separately to breeders, so for testing seedlings any desired race can be used. For example it is possible to select exclusively for resistance to *beta* and *gamma*, or to *alpha*, *beta* and *gamma* strains. In this way it is possible to carry out the selection work in stages, the advantage being that breeding material is not reduced to such an extent that other selection procedures have to be practised on a limited scale.

(f). *Rust. Uromyces phaseoli (Pers.) Wint var. typica Arthur*

In the areas where pole beans are grown, rust annually causes great damage, and summer or late crops of susceptible dwarf snap beans may often fail in these regions as a result. Damage is done through the development of numerous postules on the leaves in which the rust-brown uredospores or brownish-black teleutospores are produced as a powdery dust. The spores are distributed by the wind and can be carried over large distances. The occurrence of numerous leaf infections on susceptible bean varieties is due to the uredospores, which cause the leaves to turn yellow and wither in the early stages. At temperatures below 16°C pustules with teleutospores are produced which can only cause infection after overwintering, e.g. on bean poles. New pustules with uredospores are developed at temperatures of 16°–28°C after germination of the first uredo-spores. The incubation time is dependent on the temperature and takes some 12–14 days.

Specialization of the fungus.

Numerous physiological races of the rust fungus have been described (8, 22). In the Netherlands at least two different races occur and the runner bean *Phaseolus coccineus* is the differentiating host plant. On assessing a number of dwarf snap beans for resistance to rust in the field it appeared, however, that the same varieties were consistently susceptible or resistant. This is promising in connection with the breeding of new rust-resistant bean varieties.

Methods for testing.

It is not yet possible for private breeders to test for resistance artificially since no rust spores can be supplied to them as yet, and also because no artificial infection method has so far been tried in the Netherlands. Testing in the field is possible by sowing beans late in the pole bean regions in the north-eastern part of the Netherlands.

3. Cultivated varieties and wild species as sources of resistance

(a). *Horticultural varieties*

Dwarf snap beans

Resistance to common and yellow bean mosaic.

In the Netherlands the late Mr. C. N. Vreeken Sr. started to select for resistance to common bean mosaic before 1940 by crossing with the tolerant Swiss dwarf snap bean Enfant de Mont Calme. From this cross he obtained different types and in 1956 he introduced the variety Prelude.

After 1940 other breeders frequently used the American dwarf snap bean, US 5 Refugee, as a source of resistance to common bean mosaic (30). It soon appeared, however, that both these varieties were not sufficiently resistant to *Phaseolus-virus 2*. In addition it subsequently appeared that US 5 Refugee was susceptible to black root which was found to be due to hypersensitivity to *Phaseolus virus 1* at temperatures over 20°C. US 5 Refugee also appeared to be susceptible to stipple streak, halo blight and anthracnose. All this led to the variety becoming less popular as a parent, not to mention its undesirable vining habit, late development and light-coloured rather

tasteless pods. In spite of this, some varieties of snap beans grown today are the outcome of crosses with US 5 Refugee, e.g. Furore, Servus, Voorluk, and Widusa which, in turn, are now being used as parent plants.

After 1945 some dwarf French beans attracted attention, for example Abondant (- Maireau) and Métis which, in the field, showed a high degree of tolerance against *Phaseolus virus 2* and were only slightly damaged by *Phaseolus virus 1*. By crossing with these varieties a fair degree of tolerance to virus diseases could be obtained, but great susceptibility to halo blight and anthracnose again formed an obstacle.

Resistance to other virus – and fungus diseases.

In the cold wet summers of 1948, 1954 and 1956 numerous bean varieties gave rise to failures as a result of attack by stipple streak, anthracnose and bean rust and, as a result, the search for resistance to these diseases was begun soon after 1948. Only the German dwarf snap bean Schreiber's Imuna seemed most resistant and it is therefore understandable why this variety became highly valued as a parent. Resistance to anthracnose in the laboratory, however, was not as good as in the field and this is because Schreiber's Imuna is very susceptible to the *delta*-race and also to the *gamma*-race in slight degree as well. In testing numerous dwarf snap beans no variety sufficiently resistant to the *delta* race was found. From the results of testing Haricot beans it had become known, however, that resistance to this race could be found in some "horticultural" beans.[1]) Besides the German variety Bo22 from the collection of Prof. dr. W. RUDORF, Köln-Vogelsang, appeared to be resistant to the *delta*-race. Later still more varieties with this resistance were found, e.g. the white-seeded Haricot bean Kaboon (which appears in the Dutch List of Varieties of Field Crops).

In 1955 the dwarf snap bean Processor came into vogue in the Netherlands because of its productiveness and also owing to its great resistance to virus diseases. In 1956 it appeared in the field that the variety was very susceptible to halo blight and anthracnose; this was already known from laboratory tests. The variety proved, however, to be resistant to rust. The consumption quality of Schreiber's Imuna and Processor is not highly appreciated, but it seems possible to select for a good quality in hybrid populations derived from crosses with them, particularly when the method of repeated backcrossing is practised. This also applies to crosses with Haricot beans.

Dwarf beans for slicing

In designing breeding programmes for dwarf beans for slicing one is faced with the difficulty that little resistance to the above-mentioned diseases is to be found in existing varieties. One is therefore compelled to cross with virus-resistant dwarf snap beans on the one hand and also to derive resistance qualities from Haricot beans on the other. Dwarf beans for slicing are rarely grown nowadays on account of the great risk of crop failure. If varieties could be raised with greater reliability this crop could become much more important, especially since these beans can reach a higher productivity level than dwarf snap beans. It would be worth while to harvest the crop mechanically if stringless and parchmentless varieties with resistance to the most important diseases could be introduced.

[1]) See Table 2.

Table 2. Survey of susceptibility and resistance of cultivated varieties of dwarf beans to several diseases (*Vatbaarheid en resistentie van stambonen*)

R = Resistant, S. = Susceptible (*vatbaar*), T = Tolerant or slightly susceptible (*tolerant of weinig vatbaar*), V = Very susceptible (*zeer vatbaar*)

Diseases / Dwarf Bean Varieties	Phaseolus virus 1		Phaseolus virus 2		Nicotiana virus 11	Pseudomonas phaseolicola	Colletotrichum lindemuthianum				Uromyces phaseoli typica
	black root	mosaic	topnecrosis	mosaic	stipple streak		alpha	beta	gamma	delta	
Snap beans (Stringless – without parchment; *stamslabonen*)											
Dubb. Witte z.dr. (a)	R	S	V	V	V	T	S	R	R	S	V
Enfant de Mont Calme	R	T	R	S	S	S	S	S	S	S	?
Prélude (b)	R	T	R	T	T?	S	S	?	?	S	S
U.S. 5 Refugee	S	R	R	S	S	S	S	S	S	S	R
Ranger	S	R	R	S	S	S	S	S	S	S	?
Furore (c)	S	R	S–T	S	S	T	S	R	R	S	V
Widusa (d)	S	R	S–T	V	S	T	S	R	R	S	T
Voorluk (e)	S	R	R	S	V	S	S	R–S	S	S	T
Luca (f)	S	R	S	S	V	S	S	R	R	S	S
Servus (d)	V	R	R	S	S–T	S	S	R–S	S	S	R
Wade	S	R	R	S	?	S	S	S	S	S	?
Topmost	S	R	R	S	S	S	S	S	S	S	?
Processor	S	R	R	T	T	S	S	S	S	S	R
Saxa	R	S	R	S	S	T	R–S	S	S	S	S
Golden May (g)	R	S	R	S	S	T	R–S	S	S	S	S
Favorit (h)	R	S	R	S	S	S	R–S	S	S	S	S?
Probator (i)	R	S–T	R	S	S	T	S	R	R	S	V
Imuna (j)	R	S–T	R	T	R	S	R	R	R	S	R
Perfect	R	V(–T)	V	V	V	R	S	R	R	S	V
Amateur (k)	R	S–T	S–T	S	V	T	S	R	R	S	V?
Mignon (l)	R	T	R	S	V	T	S	R	R	S	?
French beans: (with strings and parchment; *vliezige stamslabonen met draad*)											
Masterpiece	R	S	R	T	R?	S	S	S	S	S	?
Abondant (Maireau)	R	S–T	R	T	R?	S	S	S	S	S	?
Métis	R	S–T	R	T	R?	S	S	S	S	S	?
Cabanais tardif	T	T	R	T	R?	S	S	S	S	S	?
Florida Belle	S–T	R	R	S	R?	S	S	S	S	S	?
Wagenaar	R	S	R	S	R?	S	S	S?	S	S	?
French beans: (for slicing; *stamsnijbonen*)											
Noordster (Nordstern)	R	V	V	V	V	T	S	R?	R?	S	V
Coco blanc nain hatif s.f.	S–T	R	R	S	S–T	T	?	?	?	?	?
Kaiser Wilhelm	R	S–T	R?	S	S	T	S	R?	R?	S	?
Haricot beans: (*Landbouwstambonen*)											
Walcherse Witte	R	S	V	V	V	R	S	R?	R?	S	?
Michelite	R	S–T	S–T	S	S–T	T	S	R	R	S	?
Robust	R	S–T	S–T	S	S–T	T	S	S	R	S	?
Great Northern	R	R	R	R	S–T	T	S	S	S	S	V
Red Mexican U 134	R	T	R	S	S–T	T	S	S	R	S	V
Kaboon (m)	R	S	R	T	S–T	S	R	R	S	R	R?
Krombek	R	S	R	T	S–T	S	R	R–S	S	S	R
Perry Marrow	R	S	R	T	T	S	R	R	S	S	R?
Wicosta (n)	R	S	R	S?	?	S	S	R	R	S	?
Bo 22 (z)	R	S	R	T?	T	S	R	S	S	R	?
Kievit ("Horticultural bean")	R	S	R	T	T	S	R	S	R–S	R	R
Strogele	R	S	S	S	S–T	S	R	S	S	R–S	?
Beka (o)	R	V	S	S	S–T	S	S	S	S	S	?
Bataaf (p)	R	S	S	S	S–T	S	S	S	S	S	?
Nanko (k)	R	S	S	S	S–T	S	S	S	S	S	?
Red Kidney (g)	R	S	R	S	?	V	R	S	S	S	?

The letters (a–z) refer to the list of breeders mentioned under table 3.

FIG. 1. STIPPLE STREAK IN LEAF AND PODS OF THE STRINGLESS DWARF SNAP BEAN, VARIETY DUBBELE WITTE

PHASEOLUS VIRUS 1

Fig. 2. Black root in the stringless dwarf snap bean, variety Servus

PHASEOLUS VIRUS 1

Fig. 3. Common bean mosaic in the dwarf haricot bean, variety Beka, originating from seed-borne virus

PHASEOLUS VIRUS 2

Fig. 4. Apical necrosis in young plant of the stringless dwarf snap bean, variety Dubbele Witte

PHASEOLUS VIRUS 2

FIG. 5. YELLOW BEAN MOSAIC IN THE STRINGLESS DWARF SNAP BEAN, VARIETY SERVUS

N. HUBBELING

PSEUDOMONAS PHASEOLICOLA (BURK.) DOWSON

FIG. 6. HALO BLIGHT IN THE STRINGLESS DWARF SNAP BEAN, VARIETY VOORLUK

FIG. 7. ANTHRACNOSE IN THE STRINGLESS DWARF SNAP BEAN, VARIETY RANGER

UROMYCES PHASEOLI
(PERS.) WINT. VAR. TYPICA ARTHUR

FIG. 8. BEAN RUST: UREDOSPORES ON LEAF AND TELEUTOSPORES ON POD OF THE STRINGLESS DWARF SNAP BEAN, VARIETY FURORE

Pole snap beans

Resistance to virus diseases.

In breeding for resistance in pole beans attention has first been paid exclusively to common bean mosaic. In 1940 the pole bean Mombacher Speck was used as a resistant parent. This variety also appeared to be hypersensitive to *Phaseolus virus 1* during warm weather. On account of its susceptibility to anthracnose and rust, and also owing to the moderate quality of the pods it soon lost its attractiveness as a parent. From crosses with Mombacher Speck two new pole beans were developed which were resistant to common bean mosaic and also produced pods of a better quality. These varieties, called Aromata and Animo, showed some resistance to stipple streak in the field.

After 1945 the white-seeded Stringless Early Blue Lake came to the fore. Its pods were of a better quality than those of Mombacher Speck but its resistance to other diseases, apart from virus 1, left much to be desired.

Resistance to fungus diseases.

In order to introduce more resistance into pole bean varieties it is necessary to cross with dwarf "horticultural" beans of the "Kievit"-type. Crosses with the dry shell bean varieties Bo 22 and Kaboon may also lead to good results. In the F_2, as well, dwarf snap beans can be selected. On account of the susceptibility to common bean mosaic and halo blight of all these parent plants it is desirable for the other varieties to be resistant to these diseases.

In growing pole snap beans it may be expected that more and more competition will be experienced from mechanically-harvested dwarf snap beans. Therefore it is desirable to raise varieties which do not develop parchment or strings, are not difficult to pick, and which produce a high yield in a short time.

Undoubtedly there will be a need for a resistant stringless pole snap bean (of the Blue Lake type) with rather long thick, soft and fleshy pods occurring in clusters. The interest taken in the Dutch stringless pole snap bean Aromata points in the same direction.

Pole beans for slicing

Resistance to virus diseases.

There are only a few breeders working on pole beans for slicing with the purpose of raising resistant varieties. However, to obtain good results crosses should be made with dwarf "horticultural" beans of the "Kievit"-type in order to secure sufficient resistance qualities. As in the case of dwarf snap beans only resistance to common bean mosaic was at first considered. In this connection use was made of the German pole bean Mombacher Speck as far back as 1940 but apart from the lack of qualities mentioned above, the thick round pods were considered undesirable, since commercial growers require a slicing variety with broad flat pods (sword type). By contrast the processing industry prefers pole beans with round or oval pods for slicing, since varieties with really flat sword-like pods generally soon develop a parchment layer. The Dutch consumer terms this bad quality „razors" and yet the flat pods used for slicing are still very popular on the fresh market.

The varieties Infra and Supra are two new pole beans for slicing which are resistant to common bean mosaic. They are both derived from crosses made between the varieties Verschoor and Mombacher Speck and were introduced together under the name Combine. However, because they have little resistance to other diseases, new parent plants were soon being sought. The German pole bean Blauhülsige Speck appeared to be slightly resistant to yellow bean mosaic and stipple streak, and from a hybrid population derived from it, the variety Bébé was raised, which possesses about the same resistance qualities. A rapid development of the parchment-layer of its almost stringless pods is still an unfavourable feature of this variety, should picking be delayed.

In view of the desirability of raising stringless and parchmentless pole beans for slicing, particularly for the processing industry, attention was drawn to the Swiss pole bean Wachs Rheingold, the more so since this variety appeared to be resistant to common bean mosaic and only slightly susceptible to yellow bean mosaic and stipple streak. On the other hand it is very susceptible to halo blight and anthracnose. The almost tasteless weak yellow pods are also not popular with breeders. Despite this, promising hybrids have been obtained with this type of wax-podded, stringless broad sword pole bean without parchment. It may be expected, therefore, that in the future stringless green and almost parchmentless pole beans for slicing with a fair resistance to diseases will find a place amongst the varieties available. By the creation of stringless and disease-resistant dwarf beans for slicing which do not develop parchment and can be harvested mechanically, only very early and very late crops of pole beans for slicing will eventually remain important.

Resistance to fungus diseases.

Before that time arrives, however, resistance to anthracnose and rust will have to be introduced by crossing. Due to lack of resistant parent material this resistance has to be sought for in "horticultural" beans or other resistant dry shell beans (see page 125). To secure greater resistance it is necessary to cross parchmentless and stringless pole beans for slicing and parchmented stringy dwarf beans. Although it is easy to make such crosses, to obtain usable beans for slicing purposes it is generally necessary to back-cross with pole beans for slicing.

An additional advantage of this kind of cross is the possibility of selecting, in the F_2, dwarf varieties of beans for slicing, and even dwarf and pole snap beans. There is undoubtedly much less chance of finding resistant dwarf snap beans of good quality in this way than in the case of mutual crosses between dwarf beans, but there is more chance of practical results than in the case of crosses with wild botanical species.

(b). *Cultivated varieties as field crops*

Dwarf haricot beans

Some breeders have carried out breeding work for a long time with dry shell beans, but interest seems to have been lost for some years owing to the poor results obtained, apart from few exceptions.

Fortunately, new prospects have appeared just in time, and breeding for resistance is now also being carried out, for example at the Foundation for Agricultural Plant Breeding (S.V.P.), Wageningen.

As a contradistinction to the breeding work on dwarf snap beans, the programme undertaken in dry shell beans did not start with breeding for resistance to virus diseases, but to halo blight. This is because in the western part of the Netherlands much halo blight occurs in these beans. The cool and moist climate and the prevailing strong winds encourage a rapid spread of bacteria in the coastal areas where most dry shell beans are grown.

The first crosses were made again with American parent varieties, namely the varieties Red Mexican U I 34, Great Northern types and Michelite. The first-mentioned variety is a small-seeded purplish-red bean of fine consumption quality with resistance to common bean mosaic and halo blight. The two other varieties are small-seeded white beans with about the same resistance qualities. These varieties are different to the current Dutch varieties in that their vining stems have numerous small leaves. They have a prolonged flowering period and produce pods late in the season so that it is often difficult to harvest seeds of good quality. Seed quality is also jeopardised by a heavy susceptibility to anthracnose.

It is not surprising, therefore, that no better varieties could be found. The selection for resistance to halo blight was carried out successfully, but resistance to anthracnose decreased. In susceptible white-seeded bean varieties the slightest attack of the seeds by *Colletotrichum* is conspicuous, so that breeding work was confined to brown beans.

In wet summers, like those of 1954 and 1956, susceptible brown beans even appear to suffer severe damage by anthracnose. One of the large breeding establishments therefore attempted to secure resistance to anthracnose by crossing with the American wild black-seeded dwarf bean Cornell 49–242 [1]). It appeared that the resistance to all the physiological races of the fungus was due to one single dominant gene, and in this case complete resistance could be easily obtained by means of repeated back-crossing. Another possibility is to use the parent plants mentioned in the section on breeding dwarf snap beans (see page 125). In breeding white-seeded dwarf beans or dwarf "horticultural" beans, use can be made particularly of dwarf snap beans as parent plants in order to secure resistance to all the races of *Colletotrichum*.

(c). *White and red runner beans*

In the Netherlands some white-flowering and white-seeded varieties of *Phaseolus coccineus* are cultivated as beans for slicing purposes. This species is extremely resistant to many diseases except to halo blight and rust (20, 21). The white-seeded runner bean is not only resistant to virus diseases and anthracnose, but also possesses reasonable resistance to cold; this is better still in the case of the red-flowering varieties.

Crosses of *Phaseolus coccineus* are made in numerous European countries. The present writer found that the segregating hybrid populations always exhibit extreme variations. Great differences in growth habit, form and colour of flowers, pods and seeds are conspicuous.

Resistance qualities also appear to vary greatly in the hybrid populations. The diseases sometimes occur in an almost unrecognizable form if resistance is presumed to be there. For instance, it was established that yellow bean mosaic can manifest itself by symptoms resembling a very slight degree of common bean mosaic. In addi-

[1] See legend of Table 3: *Phaseolus vulgaris nanus virides* (v).

tion the symptoms of *Phaseolus virus 2* sometimes take the form of numerous small yellow dots in the leaves. In other cases stipple streak caused a red discoloration in the nodes but the pods were not usually attacked for another month. In yet another case an attack of the roots by stipple streak appeared to occur; this produced a slight degree of yellowing and withering of the leaves, and did not allow the virus to be identified in these parts.

Resistance to all four different physiological races of *Colletotrichum lindemuthianum* soon appeared to occur only in a few plants. For a practical breeder it is not simple, therefore, to select resistant varieties, which are important for cultivation, by crossing with runner beans. Although a sufficient degree of disease resistance can be achieved in a different way, runner beans remain important as parent plants on account of their resistance to cold.

(e). *Lima bean. Phaseolus lunatus*

The lima bean *Phaseolus lunatus* is unknown in the Netherlands but represents an important crop in America and Southern Europe. Although this species probably does not surpass the runner bean so far as disease resistance is concerned, it requires such a high temperature that its cultivation in the open is impossible in the Netherlands.

(f). *Wild botanical Phaseolus species*

Phaseolus dumosus

A botanical species which has been used as a parent plant in the Netherlands on a

FIG. 11. LEAVES, WHITE FLOWERS AND POD OF PHASEOLUS DUMOSUS
Bladeren, witte bloemen en peul van Phaseolus dumosus

Fig. 12

Fig. 13

FIG. 12. RED FLOWERING TYPE OF PHASEOLUS COCCINEUS FORMOSUS WITH SMALL LEAVES AND PODS
Rood bloeiend type van Phaseolus coccineus formosus met kleine bladeren en peulen

FIG. 13. SMALL ROUNDED LEAVES, VIOLET FLOWER BUDS AND SMALL PODS OF PHASEOLUS RETUSUS METCALFII
Kleine afgeronde bladeren, violette bloemknoppen en kleine peulen van Phaseolus retusus metcalfii

limited scale is *Phaseolus dumosus* (7, 8). Its resistance and seed qualities parallel those of the runner bean although the species is less cold-resistant. A disadvantage of this species is that it flowers and sets pod only with short day length. Its consumption qualities seem to be more similar to those of *Phaseolus lunatus* than to those of *Phaseolus vulgaris* and *Phaseolus coccineus*. Yet from crosses with dwarf snap beans and dwarf beans for slicing earlier types were obtained which resembled *Phaseolus vulgaris* in numerous qualities. Good resistance to virus diseases and anthracnose was achieved.

Small seeded Phaseolus species

Apart from *Phaseolus dumosus*, real small-seeded wild species were collected in Guatamala and Mexico by OLIVER NORVELL, of the Carnegie Institute at Palo Alto (California, U.S.A.), and, among other species, Ir. R. P. LAMMERS brought back the following species from this collection to the Netherlands: *Phaseolus vulgaris* (wild), *Phaseolus coccineus* var. *striatus*, *Phaseolus coccineus* var. *formosus*, *Phaseolus retusus* forma *metcalfii* and *Phaseolus filiformus*. The resistance qualities of these species are set out in table 3. From Prof. dr. W. RUDORF, Köln-Vogelsang, we received some

TABLE 3. SURVEY OF SUSCEPTIBILITY AND RESISTANCE OF CULTIVATED POLE BEAN VARIETIES AND WILD PHASEOLUS-SPECIES TO SEVERAL DISEASES (*Vatbaarheid en resistentie van gecultiveerde stokbonen en wilde Phaseolus-soorten*)

R = Resistant, S = Susceptible (*vatbaar*), T = Tolerant or slighty susceptible (*tolerant of weinig vatbaar*), V = Very susceptible (*zeer vatbaar*)

Diseases / Pole Bean Varieties	Phaseolus virus 1: black root	Phaseolus virus 1: mosaic	Phaseolus virus 2: topnecrosis	Phaseolus virus 2: mosaic	Nicotiana virus 11: stipple streak	Pseudomonas: phaseolicola	Colletotrichum lindemuthianum: alpha	Colletotrichum lindemuthianum: beta	Colletotrichum lindemuthianum: gamma	Colletotrichum lindemuthianum: delta	Uromyces phaseoli typica
POLE SNAP BEANS: (without parchment; *stokslabonen*)											
Westlandia (r)	R	S	V	V	V	T	S	R	R	S	S
Aromata (c)	T–S	R	S	S	T	T	S	S	S	S	V
Phenomen	R	T	S–T	S	S	T	S	S	S	S	S
Animo (c)	S	R	S	S	S	T	S	S	S	S	V
Mombacher Speck	S	R	S	S	S	T	S	S	S	S	V
Early Blue Lake (s)	S	R	S	S	S	T	S	S	S	S	V
POLE BEANS FOR SLICING: (with parchment and strings; *stoksnijbonen met draad*)											
Verschoor	R	S	S	S	S	T	S	R	T	S	S
Allervroegste	R	T–S	S–T	S	S	T	S	T	R	S	S
Sabre de Malines	R	T	T	S	V	T	S	R	T	S	V
Combine (c)	S	R	S	S	V	T	S	S–T	T	S	V
Romore (c)	R	T?	T–S	S	V	T	S	S	R	S	?
Superba (t)	R	T?	S–T	S	V	T	S	S	R	S	?
Bébé (u)	R	T	R	T	T	T	S	S	T	S	V
Blauhülsige Speck	R	T	R	T	T	T	S	S	S	S	?
POLE BEANS FOR SLICING: (with or without parchment, stringless; *stoksnijbonen zonder draad*)											
Veense z.dr.	R	S	S	S	S	T	S	S–T	T	S	?
Wachs Rheingold	R	R	T	S–T	T	S	S	S	S	S	?
SCARLET RUNNER BEANS (Phaseolus coccineus; *roodbloeiende pronkbonen*)											
Prizewinner	R	R	R	R	R	S	R	R	R	R	?
Scarlet Emperor	R	R	R	R	R	S	R	R	R	R	?
WHITE RUNNER BEANS: (Phaseolus coccineus; *witbloeiende pronkbonen*)											
Emergo	R	R	R	R	R	S	R	R	R	R	S
Erecta (b)	R	R	R	R	R	S	R	R	R	R	S
LIMA BEANS (Phaseolus lunatus)											
Italian pole lima bean	R	R	R	T	R	T	R	R	R	R	?
WILD PHASEOLUS SPECIES: (*wilde Phaseolus soorten*)											
P. vulgaris aborigineus (z)	R	R	R	R	?	T	R	R–T	T	R	?
P. vulgaris nanus virides (v)	R	R	S	S	S	T–S	R	R	R	R	R?
P. cocc. formosus (w)	R	R	R	R	R	S	R	R	R	R	R?
P. cocc. formosus (x)	R	R	R	R	S	T	R?	R?	T?	T?	R?
P. dumosus (y)	R	R	R	R	R	S	R	R	R	R	R?
P. retusis f. metcalfii (y)	R	R	R	R	R	R	R	R	R	R	R?
P. filiformis (y)	R	R	R	R	S	R	R	R	R	R	R?
Estimated number of genes at least required for resistance	1*r*(*1*)	1*d*(*2*) 1*r*(*1*)	1*r*(*4*)	1*r*(*4*)	2*r*?	2*r*(*25*)	2*d*(*23*)	2*d*(*23*)	1*r*, 2*d*(*23*)	2*d*(*3*)	1*d*?, 2*r*?(*36*)

The numbers in italic type correspond with list of references; *d* = dominant; *r* = recessive

seed of *Phaseolus vulgaris* f. *aborigineus*. From Dr. BECKER, Wybelsum, we received a black seeded type of *Phaseolus vulgaris nanus virides* with violet pods. This type originated from the Department of Plant Breeding at Ithaca (N.Y.) (see Table 3).

Although resistance to the most important diseases exists in the foregoing wild species, it has not so far been possible to use them in cross-breeding. In general they are small-leaved pole beans with small seeds. The seeds of *Phaseolus filiformus* are hardly more than 1 mm in length, and are so heavily wrinkled that they would not be recognised as being a bean species. *Phaseolus retusis* forma *metcalfii* seldom produces pods and seeds in the Netherlands. The form and size of the seeds resemble soybeans.

From the above it is clear that much labour is involved in transferring other resistance qualities from botanical *Phaseolus species* into cultivated varieties. However, it is with much interest that the results of the breeding work being carried out with *Phaseolus dumosus* as a parent at the Institute of Horticultural Plant Breeding (I.V.T.), Wageningen, and the Vegetable Research Station, Alkmaar, are awaited.

4. Difficulties and possibilities in selecting for resistance against several diseases

(a). *Genetical basis of combined resistance*

In table 2 and 3 a survey is given of resistance against six different diseases of some important cultivated bean varieties and parent plants used in the Netherlands. Since it is often not possible to combine all the desired resistance qualities by means of a single crossing, it is always desirable to use parent plants which are resistant to several diseases. Combining resistance to a number of diseases implies the combining of a fairly large number of genes governing resistance. The actual number can be partially determined although it may vary according to the particular parent plant involved. A rough estimate of the number of genes governing resistance in most of the parents is given in table 3. This number of estimated factors totals 20 and when all these factors segregate freely in the F_2 of a crossing, $(2^{20})^2 = \pm$ 1,000,100,000,000 gene-combinations are possible. This astronomical number has already been quoted by Erwin BAUR (5) in connection with flower characters in a cross between *Antirrhinum majus* and *A. molle*. If it is assumed that an area of 1 ha is required for growing 100,000

Legend of table 3.

(a) = Princesse s.f. = Dopp. holl. Prinzess
(b) = Vreeken's Zaden, Dordrecht
(c) = Rijk Zwaan, Rotterdam
(d) = Institute of Horticultural Plant Breeding, Wageningen (N. Hubbeling)
(e) = Vegetable Research Station, Alkmaar (Tj. Buishand)
(f) = Nunhem, Haelen, L.
(g) = P. Koomen, Spanbroek
(h) = Van Waveren & De Bres, Hannover-Münde
(i) = Wed. P. de Jongh, Goes
(j) = Dr. Schreiber, Braunschweig
(k) = Sluis en Groot, Enkhuizen
(l) = A. R. Zwaan en Zn., Voorburg
(m) = Joordens, Blerick (Venlo)
(n) = Coöp. Zaaizaadvereniging, Wijdenes
(o) = Centraal Bureau, Rotterdam
(p) = D. J. v. d. Have, Kapelle-Biezelinge
(q) = Michigan Dark –
(r) = Gebr. v. d. Berg, Naaldwijk
(s) = Stringless –, whiteseeded = Juli o.F.
(t) = Ezetha's –: Enkhuizer Zaadhandel, Enkhuizen
(u) = Bulten's Zaden, Aalten
(v) = Obtained from Dep. of Plant Breeding, Ithaca (New York) via Dr. Becker, Wybelsum (W. Deutschland). Seems to be identical with Cornell Resist. Dry Shell Bean 49–242
(w) = Red flowering type, obtained from O. Norvell, Carnegie Institute, Palo-Alto (California) via Ir R. P. Lammers, Wageningen
(x) = Violet flowering type, same origin
(y) = Same origin as (w) and (x).
(z) = Prof. Dr W. Rudorf, Köln-Vogelsang

TABLE 4. PROCEDURES USED IN BREEDING FOR RESISTANCE TO FIVE DISEASES

DWARF SNAP BEANS:	
	Dubbele Witte z.dr. × Kaboon Processor × Widusa
	HBC × YAD MYS × MHBC
Selection in (F_2), F_3, F_4, (F_5) Crossing of F_5 or F_6 lines	*YHABCD* × M*YSHBC*
Selection in F_2 en F_3 only for resistance to common bean mosaic, stipple streak, alpha and delta races of *Colletotrichum*	MYSHABCD
DWARF AND POLE BEANS FOR SLICING:	
	Noordster × Kievit Wachs Rheingold × Veense z.dr.
	H × YSAD M × HBC
Selection in (F_2), F_3, F_4, (F_5) Crossing of F_5 or F_6 lines	*HYSAD* × M*H*BC
Selection in (F_2), F_3, F_4, (F_5) Intercrossing of F_5 or F_6 lines	*SHABCD* × MY*HABCD*
Selection in F_2 and F_3 only for resistance to virus diseases	MYSHABCD
POLE SNAP BEANS:	
	Aromata × Kaboon Westlandia × Phenomeen
	MSH YAD HBC MH
Selection in (F_2), F_3, F_4, (F_5) Crossing of F_5 or F_6 lines	*MYSHAD* × *MH*BC
Selection in (F_2). F_3, F_4, F_5 only for resistance to yellow bean mosaic, stipple streak and anthracnose	MYSHABCD

Indication of resistance to [1]):

Common bean mosaic	M	*Colletotrichum lindemuthianum*	alpha	A
Yellow bean mosaic	Y		beta	B
Stipple streak	S		gamma	C
Halo blight	H		delta	D

bean plants, one billion one hundred million plants would require an area three times the size of the Netherlands. An area of more than 10 ha would be required for all the homozygotes which could result after segregation. ($2^{20} = 1{,}048{,}568$). Even in that case, selection for resistance would be virtually impossible.

(b). *Inheritance of resistance*

Since resistance for several diseases are inherited recessively, many undesirable combinations are produced from which more resistant plants may emerge subsequently, and testing the F_2, in such cases, may mean the loss of valuable material. On

[1]) Capitals in italic: No selection necessary in double cross populations for resistance to the concerning diseases.

the other hand, with dominant inheritance of resistance a segregation of susceptible plants in the offspring should be allowed for, and the homozygously-resistant plants should be selected by means of testing. It may therefore be of importance to harvest plants separately from the F_2 generation onwards. However, it is impossible to combine at once all desired features in one line and one should be satisfied to start with a rather small number of genes.

The following shows the number of undesirable plants which would segregate freely for every single plant of a desirable type, as the number of dominant or recessive genes in the F_2 increases:

Number of factors	1	2	3	4	5	6	7	8
Number of undesired plants by the side of 1 single desired plant in case of:								
Dominance	$^1/_3$	$^7/_9$	$^7/_5$	2	3	5	7	10
Recessiveness	3	15	63	255	1,023	4,095	16,383	65,535

It follows that it is not difficult to combine at least eight genes in the F_2 where the resistance is dominant. In that event one should think in terms of testing a large number of lines for three consecutive generations in order to find one homozygously-resistant line.

If the resistance is recessive it is necessary to select for resistance in the offspring of many susceptible plants in order to obtain a sufficient number of plants with the desired genetical constitution, for the survey clearly shows that in an F_2 population of 1,000 plants there is little chance of finding a single resistant plant with at least 5 recessive resistance genes.

(c). *The procedure of combined resistance testing*

In carrying out the practical work of consecutive testing for resistance against the above-described diseases it appears to be easier to obtain combined resistance than would be expected in theory. Among other reasons, this can be attributed to the resistance not being absolutely dominant or recessive.

When the plants are attacked by anthracnose, all transitional stages from healthy to heavily attacked can be observed in the affected plants and this is undoubtedly associated with incomplete dominance. With other diseases as well, such as yellow bean mosaic, stipple streak and halo blight, resistance is presumably inherited recessively as a rule, and clear differences in attacks on susceptible plants are observable.

The present writer found from experience that F_1 plants resulting from crosses between susceptible and resistant varieties are less susceptible than the susceptible parent plants. In the hybrids which contain the resistance qualities in heterozygous form, no dying off is observed as may be the case in homozygously-dominant susceptible plants. In consequence there is little objection to testing for some tolerance against these diseases in the F_2. In point of fact in the F_3, F_4 etc. plants with some tolerance can be selected as well as ever-increasing numbers of resistant plants, which continue to segregate.

Combined artificial testing.

Hence the procedure of selection basically involves the artificial testing of the seedlings in the F_2 for resistance to anthracnose and then assessing the remaining resistant plants directly for resistance to stipple streak and halo blight.

In a small F_2 population this testing results in the use of so much of the available material that testing for resistance against common bean mosaic and yellow bean mosaic can better be postponed until the F_3. In this case the remaining resistant F_2 plants should be increased, preferably under glass in order to prevent infection with Phaseolus virus 1 and 2 by aphids. This procedure fits in well with the method of growing two or three generations per year under glass.

Testing partly in the field.

The remaining plants of large F_2-populations can be exposed to infection in the field with *Phaseolus virus 1* and *2*, after inoculation with infection material of anthracnose, stipple streak and halo blight. If a limited amount of hybrid material is to be tested for resistance against other virus diseases in the field, the testing for resistance to stipple streak and halo blight should be postponed till the F_3. In the F_3 and F_4 it is preferable to have a large number of lines kept separate in order to be able to ascertain which resistance factors have been combined. In the F_5 and F_6 the testing should be continued with the lines in which an insufficient number of homozygously-resistant forms occur.

(d). *Intercrossing of lines*

It is not easy to find lines which possess reasonably good quality and which, at the same time, are resistant to the five diseases mentioned above. Sometimes there are a sufficient number of usable lines e.g. with resistance to anthracnose, halo blight and common bean mosaic, combined either with resistance to stipple streak or to yellow bean mosaic. It is then possible, by the intercrossing of lines and by subsequent testing for the two last-mentioned diseases, to secure resistance against five diseases in the consecutive hybrid populations.

In this case it may be wiser to start from the beginning with two different crosses. From one cross, for instance, resistance against common bean mosaic, yellow bean mosaic, stipple streak, halo blight and the *beta* and *gamma* races of *Colletotrichum* can be obtained at most by selection. From the other crosses, lines can be obtained which are resistant to yellow bean mosaic, halo blight, stipple streak and against all the races of *Colletotrichum*. It is clear that in this instance the testing for resistance against anthracnose in these crosses should be carried out separately in order to prevent one of the two crosses falling a victim to attack by the *alpha* and *delta* races of this disease (see Table 4).

In conclusion it can be stated that it is not difficult to ultimately increase the number of resistance genes by purposeful resistance testing from the F_2 onwards to such an extent that plants can be selected with at least resistance to 4 diseases. In order to be able to reach resistance to 5 diseases at least the two F_5 or F_6 lines should be intercrossed as they both contain mainly the same resistance genes. The few lacking resistance genes of one line should be compensated by resistance genes of the other and vice versa.

Summary

Since there is now sufficient evidence regarding the symptoms of the most important bean diseases in Western Europe, these can serve as a basis for resistance testing. This study led to the development of testing methods which are useful for private breeders.

The resistance department of the Institute for Phytopathological Research (I.P.O.) gives information on this subject and supplies the infection material required.

Resistance-genes were traced by testing numerous cultivated varieties and wild species. In addition, more data became available concerning the inheritance of resistance, after testing numerous crosses at private breeding establishments and official plant breeding institutes.

The following diseases and testing methods are described:

1. *Common bean mosaic and black root. Phaseolus virus 1*

Testing can be carried out by sowing in the second half of May or in the beginning of June. Seeds, infected with virus, should be sown next to beans to be tested, while a sufficient number of aphids should be available for the transmission of the virus. In the coastal areas of the Netherlands aphids are far less numerous, so it is generally necessary to test artificially by sap inoculation of the seedlings. Black root can only be transmitted in this way at temperatures above 30 C. Grafting of diseased material on to the plants to be tested is too laborious for private breeders, at least for the time being. Testing in the field for resistance to black root should be carried out all the same in the southern, central and eastern parts of the country.

2. *Yellow bean mosaic. Phaseolus virus 2*

When a sufficient number of winged aphids is available, testing can be carried out by sowing beans next to rows of infected gladioli in the second half of May or in early June. Artificial inoculation of seedlings can be brought about by the use of sap.

3. *Stipple streak. Nicotiana virus 11*

Apart from sowing in infested soil the plants can be tested by inoculating seedlings with diluted sap from diseased plants. A healthy and vigorous root system can be the proof of resistance provided that the aerial parts of the plant develop no disease symptoms.

4. *Halo blight. Pseudomonas phaseolicola (Burkh.) Dowson*

Seedlings or pods can be tested for resistance by four inoculation methods with a bacterial suspension of the causal organism. The most reliable estimation of resistance is based on the development of "greasy" spots on leaves and pods. The development of chlorotic "halo spots" is mainly dependent on a temperature of below 20 C since they are not caused by the bacteria themselves but by toxins which they excrete and which are apparently sensitive to temperature.

5. *Anthracnose. Colletotrichum lindemuthianum (Sacc et Magn.) Scrib.*

Seedlings can be tested in tubes with cotton wool plugs by inoculating with a suspension which, according to the resistance to be expected, contains spores of the

different physiological races (*alpha*, *beta*, *gamma* and *delta*). In order to allow the spores to germinate the inoculated plants are kept at 100% humidity for three days, maintaining a temperature of between 14°C and 22°C. Trays or boxes with seedlings are sealed with glass or plastic. Three days after inoculation the seal can be removed but the temperature should not vary much above or below the range mentioned. By potting up all plants tested after three days there is little risk of poor growth in consequence of bad root development. This is often the case when planting is done after the appearance of disease symptoms, especially when the beans are planted under field conditions.

From table 3 it appears that it is often not possible to combine a sufficient number of resistance genes by making a single cross. Resistance to the *delta* race of Colletotrichum should be derived from *hard shell beans* which are partly susceptible to races against which resistance is easily to be found in varieties used in horticulture.

Apart from aiming at complete resistance to anthracnose, new prospects of resistance breeding are possible through the inheritance of resistance. It appears that some 20 genes should be combined to attain resistance to the most important diseases including rust, for which so far in Europe no resistance-test has been evolved. Allowing these 20 genes to segregate freely would require an area three times that of the Netherlands in order to find a single plant with the desired features. By purposeful resistance-testing from the F_2 onwards, the number of resistance genes can be raised gradually to such an extent that plants can be selected in which at least resistance to 4 diseases occurs. In order to reach resistance to 5 diseases, which is provisionally aimed at as a maximum, two lines of the F_5 or F_6 with comparable resistance factors should be crossed reciprocally. The lacking resistance genes of one of the lines should be compensated by resistance genes of the other line and vice versa.

ACKNOWLEDGEMENT

The writer wishes to express his sincere thanks to Mr. J. D. REYNOLDS, Director of Research of the Pea Growing Research Organisation, Pea Research Station, Yaxley, Peterborough, England, for the correction of this manuscript.

Thanks are also due to the Institute of Horticultural Plant Breeding (I.V.T.) for the courtesy to supply the photographs of some botanical *Phaseolus* species.

SAMENVATTING

Nieuwe aspecten omtrent het kweken op ziekteresistentie van bonen (*Phaseolus vulgaris L.*)

Aangezien thans voldoende zekerheid bestaat over de symptomen van de belangrijkste bonenziekten in W.-Europa kunnen deze als basis voor de resistentietoetsing dienen. Daarbij werden toetsmethoden ontwikkeld, die bruikbaar zijn voor particuliere kwekers. De resistentieafdeling van het I.P.O. verschaft hierover voorlichting en levert het benodigde infectiemateriaal.

Door toetsing van talrijke cultuurrassen en wilde geniteurs konden resistentiegenen worden opgespoord. Bovendien kwamen meer gegevens beschikbaar over erfelijkheid

van de resistentie na de toetsing van tal van kruisingen bij particuliere kweekbedrijven en officiële veredelingsinstellingen. De toetsmethoden en ziekten, die hierboven werden behandeld zijn de volgende:

1. *Rolmozaïek en zwarte-vaatziekte. Phaseolus virus 1*

Toetsing kan geschieden door uitzaai in de tweede helft van mei of in het begin van juni. Naast de te toetsen rijen bonen dienen dan met virus besmette zaden te worden uitgezaaid, terwijl voldoende gevleugelde bladluizen voor het virustransport dienen te zorgen. Dit laat in de kuststreken vaak te wensen over, zodat daar kunstmatige toetsing door sapinoculatie van kiemplanten nodig kan zijn. Op deze wijze kan zwarte-vaatziekte alleen worden overgebracht bij temperaturen boven 30°C. Enting van zieke plantedelen op te toetsen planten is vooralsnog voor particuliere kwekers te omslachtig, zodat toetsing te velde op resistentie tegen zwarte-vaatziekte in het zuiden, centrum en oosten van het land dient te geschieden.

2. *Scherpmozaïek en topsterfte. Phaseolus virus 2*

Te velde kan bij aanwezigheid van voldoende gevleugelde bladluizen gemakkelijk getoetst worden door uitzaai in de tweede helft van mei of in juni naast rijen besmette gladiolen. Kunstmatige toetsing kan geschieden door middel van sapinoculatie van kiemplanten.

3. *Stippelstreep. Nicotiana virus 11*

Behalve door uitzaai in besmette grond kan gemakkelijk getoetst worden door inoculatie van kiemplanten met sterk verdund sap van zieke planten. Een gezond en krachtig wortelstelsel kan de bevestiging zijn van resistentie, indien de bovengrondse plantedelen geen ziektesymptomen ontwikkelen.

4. *Vetvlekkenziekte. Pseudomonas phaseolicola (Burkh.) Dowson*

Kiemplanten en peulen van bonen kunnen door 4 inoculatie-methoden met een bacteriesuspensie van de ziekteverwekker worden getoetst op resistentie. De betrouwbaarste beoordeling van de resistentie is gebaseerd op de ontwikkeling van vetvlekken op bladeren of peulen. De ontwikkeling van chlorotische "halo vlekken" is sterk gebonden aan een temperatuur, die beneden 20° ligt, aangezien deze niet door de bacteriën zelf, maar blijkbaar door temperatuurgevoelige toxinen (door hen afgescheiden) worden veroorzaakt.

5. *Vlekkenziekte. Colletotrichum lindemuthianum (Sacc et Magn.) Scrib.*

De toetsing van kiemplanten geschiedt in glazen buizen met wattenproppen door inoculatie met een suspensie, die naar gelang van de te verwachten resistentie sporen bevat van physiologische rassen behorende tot de onderscheiden groepen *alpha*, *beta*, *gamma* en *delta*. Teneinde de sporen tot ontkieming te brengen, worden de geïnoculeerde planten 3 dagen bij 100 % vochtigheid gehouden, waarbij de temperatuur tussen 14° en 22°C dient te blijven. Zaaitesten of kistjes worden met glas of plastic afgesloten. Drie dagen na de inoculatie kan de afdekking worden verwijderd, maar dient de temperatuur niet ver boven of onder het genoemde traject te stijgen of te dalen. Door na drie dagen alle getoetste planten op te potten, loopt men het minste risico een slechte groei tengevolge van een slechte ontwikkeling van het wortelstelsel

te krijgen. Bij uitplanting na de ontwikkeling van ziektesymptomen is dit dikwijls het geval, vooral indien te velde wordt uitgeplant.

Uit tabel 3 volgt, dat het dikwijls niet mogelijk is door het uitvoeren van een enkelvoudige kruising voldoende resistentiegenen te combineren. De resistentie tegen *delta*-physio's van *Colletotrichum* dient ontleend te worden aan enige hardschillige landbouwstambonen, die ten dele vatbaar zijn voor physio's, waartegen resistentie in het tuinbouwsortiment van bonen gemakkelijk is te vinden.

Behalve met de aldus te verkrijgen volledige resistentie tegen vlekkenziekte worden nieuwe perspectieven van de resistentieveredeling geopend door de erfelijkheid van de resistentie. Het blijkt, dat men naar schatting 20 genen bijeenbrengen moet om resistentie tegen de belangrijkste ziekten te verkrijgen, meegerekend de roest van bonen, waarvoor in Europa nog geen resistentietoets beproefd werd. Bij een vrije splitsing van deze genen zou men een oppervlakte van 3 × Nederland nodig hebben om 1 enkele plant van de gewenste hoedanigheid te vinden. Door doelbewuste resistentietoetsing van de F_2 af kan echter het aantal resistentiegenen geleidelijk zodanig worden opgevoerd, dat planten kunnen worden geselecteerd waarin ten minste resistentie tegen 4 ziekten voorkomt. Om het voorlopig gestelde maximum van resistentie tegen 5 ziekten te kunnen bereiken dienen 2 lijnen van de F_5 of F_6 onderling te worden gekruist, die grotendeels al dezelfde resistentie bezitten. De nog ontbrekende resistentiefactoren van de ene lijn moeten echter worden gecompenseerd door resistentiegenen van de andere en omgekeerd.

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